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Obesity

Effects of High-Fat Feeding on Ectopic Fat Storage and Postprandial Lipid Metabolism in Mouse Offspring

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Objective: Parental high-fat feeding was proposed to negatively impact metabolic health in offspring. Here, the ectopic fat storage in heart and liver in offspring was investigated, and the effects on mitochondrial function, *de novo* lipogenesis, and postprandial lipid metabolism were explored in detail.

Methods: Male and female mice received either a high-fat (HF) or standard chow (LF) diet during mating, gestation and lactation. All offspring animals received the HF diet.

Results: Abdominal visceral adipose tissue tended to be higher in HF/HF mice. Cardiac lipid content was also higher in the HF/HF mice (LF/HF vs. HF/HF: 1.03% ± 0.08% vs. 1.33% ± 0.07% of water signal, $P = 0.01$). In contrast, hepatic lipid content tended to be lower in HF/HF mice compared to LF/HF mice. A severely disturbed postprandial lipid clearance was revealed in HF/HF mice by the results from the triglyceride (TG) tolerance tests (LF/HF vs. HF/HF: 6,753 ± 2,213 vs. 14,367 ± 1,978 mmol l^{-1} min^{-1}, $P = 0.01$) and ^{13}C-fatty acid retention test (LF/HF vs. HF/HF: 2.73% ± 0.85% vs. 0.89% ± 0.26% retention from bolus, $P = 0.04$), which may underlie the lower hepatic lipid content.

Conclusions: Here it is shown that HF diet negatively impacts postprandial TG clearance in offspring and results in an overall metabolic unfavorable phenotype and ectopic lipid deposition in the heart and in visceral storage sites.

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Introduction

In spite of large-scaled efforts to promote a healthy body weight, the prevalence of obesity and metabolic syndrome is still rising (1), thereby increasing the risk for diabetes (2) and cardiovascular disease. It is unresolved to what extent this dramatic increase is accounted for by changed dietary habits. An underestimated factor that may exacerbate the effects of an unhealthy lifestyle may be the presence of obesity in the previous generation. There is evidence for a carry-over effect from one generation to the next. For instance, positive relationships between maternal pregravid BMI and birthweight (3), fetal insulin resistance (4), and fetal body fat percentage have been reported (5). Moreover, children of mothers with obesity have increased risk to develop metabolic syndrome (6) and congenital heart defects (7). More specifically, in non-human primates it was found that maternal exposure to a high-fat diet (31.6 energy%) resulted in a metabolic syndrome phenotype in 60% of the offspring and increased the risk for non-alcoholic fatty liver disease (NAFLD) (8). It also was shown in a rodent model that increasing the dietary fat content during pregnancy and lactation suffices to adversely affect the offspring in terms of promoting hepatic lipid storage and inflammation and diminishing markers of mitochondrial function and *de novo* lipogenesis (9). When examining lipid metabolism, most studies are limited to the investigation of fasted concentrations of plasma metabolites. However, postprandial lipid metabolism is also an important player in mediating

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metabolic risk, which is not necessarily reflected in fasted plasma concentrations. We therefore investigated how the postprandial response to a lipid load is modified in offspring of high-fat-fed mice. We investigated the effect on muscle, cardiac, and hepatic steatosis and function using non-invasive techniques. We report that offspring of mice that were exposed to an obesogenic high-fat diet have increased visceral adipose tissue (VAT) mass and increased cardiac lipid content, while hepatic lipid content tended to be lower due to delayed postprandial clearance of dietary triglyceride (TG).

Methods

Animal model and study set up

The Institutional Ethics Committee on Animal Welfare approved all experiments. C57Bl/6 mice (breeding couples from Charles River) were housed under standard conditions at 25°C with a 12:12-h light-dark cycle with ad libitum access to water and diet. Mice were randomly assigned to either a high-fat (HF) diet (45% kcal fat of which 31% saturated, 36% mono-unsaturated, 33% poly-unsaturated fatty acids, 20% kcal protein, 35% kcal carbohydrate, Research Diets, New Brunswick, NJ) or standard chow (LF) diet (9% kcal fat, 33% kcal protein, 58% kcal carbohydrate, ssniff Spezialdiäten GmbH). Mice were fed their assigned diet at least 6 weeks before mating and the diet was continued during gestation and lactation. Weight gain and food intake were measured weekly (Supporting Information Figure S1). Littersize was similar in both groups. At weaning, all offspring was fed the HF diet, generating two experimental groups: HF/HF and LF/HF. Body weight and food intake was determined each week and was similar in both groups (Supporting Information Figure S2). Measurements were only performed in male offspring, because of the possibility of sexual dimorphism. Male offspring (n=13 from each group) underwent a glucose tolerance test (GTT) at 12 weeks of age, and cardiac function and cardiac -, hepatic - and abdominal visceral fat was measured at 15 weeks of age. In a parallel group of mice, cardiac and hepatic mitochondrial function was determined at 12 weeks of age.

A third, parallel group of male offspring ($n = 9$ HF/HF and $n = 9$ LF/HF) underwent a triglyceride tolerance test (TTG) at 10 weeks of age and cardiac and hepatic lipid retention and *de novo* lipogenesis (DNL) was measured at 12 weeks of age. The study design is also presented in the Supporting Information Figure S3. Changes in ectopic fat deposition during early adulthood (around 15 weeks) have been reported before in mice when the previous generation was exposed to HF feeding (9). To study underlying mechanisms, we performed detailed measurements in mice around 10-15 weeks of age.

Magnetic resonance imaging and spectroscopy in vivo

Cardiac function, cardiac-, hepatic-, and abdominal visceral fat content were measured using magnetic resonance imaging (MRI) and spectroscopy (MRS). Mice were anesthetized with 2% isoflurane and 0.4 l min^{-1} of O_2 and placed in a cradle with warm waterbed, in a quadrature volume coil (ø 35 mm, transmit-receive, Bruker Biospin) of 7 Tesla MR System (Bruker Biospin GmbH, Ettlingen, Germany).

Visceral fat content

Coronal T1-weighted spin-echo images (matrix size: 256 × 256, FOV: 50 × 50 mm^2, TE: 9.1 ms, TR: 500 ms, NA: 1, slice thickness: 1 mm) were obtained. Using in-house developed software, VAT content was determined in the three-dimensional region from the middle of the left kidney to the middle of the bladder. Images were checked individually and manually edited in MRIcron (10).

Cardiac and hepatic lipid content assessed by ^{1}H-MRS

Cardiac ^{1}H-MRS was performed using cardiac-triggered and respiratory-gated point resolved spectroscopy (PRESS, TE = 9.1 ms, TR = 2,000 ms). Spectra were acquired from a 4-μl voxel in the interventricular septum. A water-suppressed spectrum was acquired (NSA = 512) as well as a reference spectrum (with non-suppressed water signal, NSA = 64). Spectra were fitted using the AMARES algorithm in jMRUI (11). The sum of the TG peaks at 1.55, 1.26, and 0.85 ppm was expressed as percentage of the unsuppressed water signal.

Hepatic lipid content was determined as described for cardiac lipid, but with a water-suppressed spectrum (NSA = 128) and water spectrum (NSA = 64) from a 18-μl voxel.

Cardiac function assessed by MRI

Bright blood cine images in four-chamber view (4CH), long axis view (LA), and two slices of short axis view (SA) were acquired [1-2 mm under the mitral valve and at the base of the papillary muscle were acquired as described before (12)]. End-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) were calculated using the modified Simpson Rule (12).

Glucose tolerance test, plasma insulin, and plasma metabolites

Mice were fasted for 6 h and injected intraperitoneally with glucose (2 mg g^{-1} body weight in 200 μl of sterile solution) as reported earlier (13).

Fasting plasma insulin levels were determined using a Meso Scale Discovery (MSD) assay (Gaithersburg, USA) and HOMA index was calculated. At 12 weeks of age, venous blood was collected in EDTA-containing tubes (1 mg ml^{-1}) after a 5-h fast and plasma TG and NEFA levels were determined (Roche, Schlieren, Switzerland and Wako Chemicals, Neuss, Germany, respectively).

Biochemical TG determination in gastrocnemicus

TG levels in gastrocnemius muscle were measured using the method of Schwartz and Wolins (14).

Mitochondrial isolation and respiration measurements

Mice were anaesthetized and killed by decapitation. Mitochondria were isolated as previously described with minor modifications (15). Briefly, heart and liver were excised and placed in ice-cold isolation buffer, rinsed, and minced on ice. Heart tissue was further digested with an enzymatic treatment (protease, Sigma). After homogenation and centrifugation, the resulting pellet was re-suspended in isolation buffer (HIB for heart and LIB without BSA for liver preparations) and centrifuged in two steps (at 800*g* and 8,500*g* at 4°C).The final mitochondrial pellet was re-suspended in HIB and homogenized. Mitochondrial protein content was

determined by Fluram assay (15). Mitochondrial respiration (heart: 0.1 mg ml^{-1} and liver: 0.25 mg ml^{-1}) was determined at 37°C in a two-chamber Oxygraph (OROBOROS® Instruments, Innsbruck, Austria) using pyruvate (5 mM) plus malate (3 mM) for heart or glutamate (5 mM) plus malate (3 mM) for liver and palmitoyl-coenzyme A (CoA) (50 μM) plus carnitine (2 mM) as substrates. ADP (450 μM) and oligomycin (1 μg ml^{-1}) were added to obtain state 3 and state 4 respirations, respectively.

Mitochondrial DNA and copy number

Mitochondrial DNA copy number was determined by the ratio of COX2 expression over UCP2 expression as described previously (16).

Hepatic *de novo* lipogensis

Fractional contribution of DNL to the hepatic lipid pool was determined using $^{1}H/^{2}H$ NMR spectroscopy of liver extracts after $^{2}H_2O$ enrichment (Sigma-Aldrich, St Louis, MO) as described before (17). Total hepatic TG and DNL fraction were quantified against pyrazine. The ratio between ^{2}H enriched body-water and the ^{2}H enrichment of hepatic TG methyl hydrogens determines the contribution of DNL to the total hepatic TG pool (18).

Cardiac and hepatic lipid retention

To determine the contribution of dietary fat to myocardial and hepatic TG, mice were fasted for 4 h and given an oral load of [U-^{13}C] algal-lipid mixture (Cambridge Isotope Laboratories, Andover, MA, 5 g kg^{-1} body weight). Four hours later, animals were killed and hearts and livers were freeze-clamped and lipids were extracted and analyzed by proton-decoupled ^{13}C-NMR spectra of cardiac and hepatic lipids as described before (17).

Dietary lipid absorption to heart or liver (% of bolus) is given as =

$$\frac{\text{total cardiac or hepatic TG } (\mu\text{mol·g-1}) \times \text{13C-enrichment}(\%) \times \text{heart or liver weight(g)}}{\text{oral lipid supply}(\mu\text{mol})}$$

Triglyceride tolerance test

Mice were shortly fasted (4 h) and received an oral bolus of olive oil (11 g kg^{-1}). Plasma TG was assessed every hour for the next 4 h with a standard TG meter (Cardiocheck, Belgium).

mRNA expression of LPL and modulators of LPL in hepatic and white adipose tissue

For analyses of mRNA of LPL, ANGPTL4, GPIHBP1, and PPARγ in mouse white adipose tissue (WAT) and mRNA of hepatic lipase, ANGPTL4, ANGPTL3, apoE, and apoC3 in liver tissue, total RNA was isolated with TRIzol reagent (Invitrogen, Breda, The Netherlands) according to manufacturer's instructions. One μg of total RNA was reverse transcribed using iScript (Bio-Rad, Veenendaal, The Netherlands). cDNA was PCR-amplified with Platinum Taq DNA polymerase (Invitrogen) on a Bio-Rad CFX384 Real Time System using Sensimix (Bioline, GC Biotech, Alphen aan de Rijn, The Netherlands). Ribosomal protein 36B4 mRNA served as control. PCR primer sequences (PrimerBank) were ordered from Eurogentec (Seraing, Belgium).

Statistical analysis

Results are expressed as means ± standard error of the mean (SEM). Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp). To evaluate effects between the two experimental groups, a two-tailed Student's t-test was used. Significance was set at $P \leq 0.05$.

Results

Body weight, organ weight, and visceral fat content

Body weight tended to be increased at 12 weeks of age (LF/HF vs. HF/HF: 25.5 ± 0.7 vs. 27.3 ± 0.7 g, $P = 0.08$) in HF/HF mice from group 1. VAT volume, also tended to be higher (LF/HF vs. HF/HF: 581.9 ± 33.8 vs. 881.2 ± 106.7 μl, $P = 0.08$, Figure 1). No difference in heart or liver weight was observed.

Fasted plasma metabolites and glucose tolerance test

Fasted plasma glucose, insulin, plasma TG and NEFA levels were similar in both dietary groups (Table 1). Therefore, HOMA-IR was also similar. Early exposure to a HF diet did not change glucose tolerance (Figure 2).

Skeletal muscle lipid content

Skeletal muscle lipid content was not affected by early exposure to a HF diet (LF/HF vs. HF/HF: 0.015 ± 0.002 vs. 0.012 ± 0.002 TG μl^{-1} protein, P = ns).

Cardiac function, lipid content, and mitochondrial respiration

Early exposure to a HF diet resulted in significantly increased cardiac lipid content at 15 weeks of age (LF/HF vs. HF/HF: 1.03% ± 0.08% vs. 1.33% ± 0.07% of water signal, $P = 0.01$, Figure 3). To investigate if changes in cardiac lipid content were associated with alterations in mitochondrial oxidative capacity, mitochondrial respiration was measured in isolated mitochondria in parallel groups. Prenatal exposure to the HF diet did not result in alterations in pyruvate or palmitoylCoA + carnitine-supported respiration or mitochondrial copy number at 12 weeks of age. These differences in cardiac lipid content furthermore did not translate into differences in cardiac systolic function (EDV, ESV and EF, Supporting Information Figure S4).

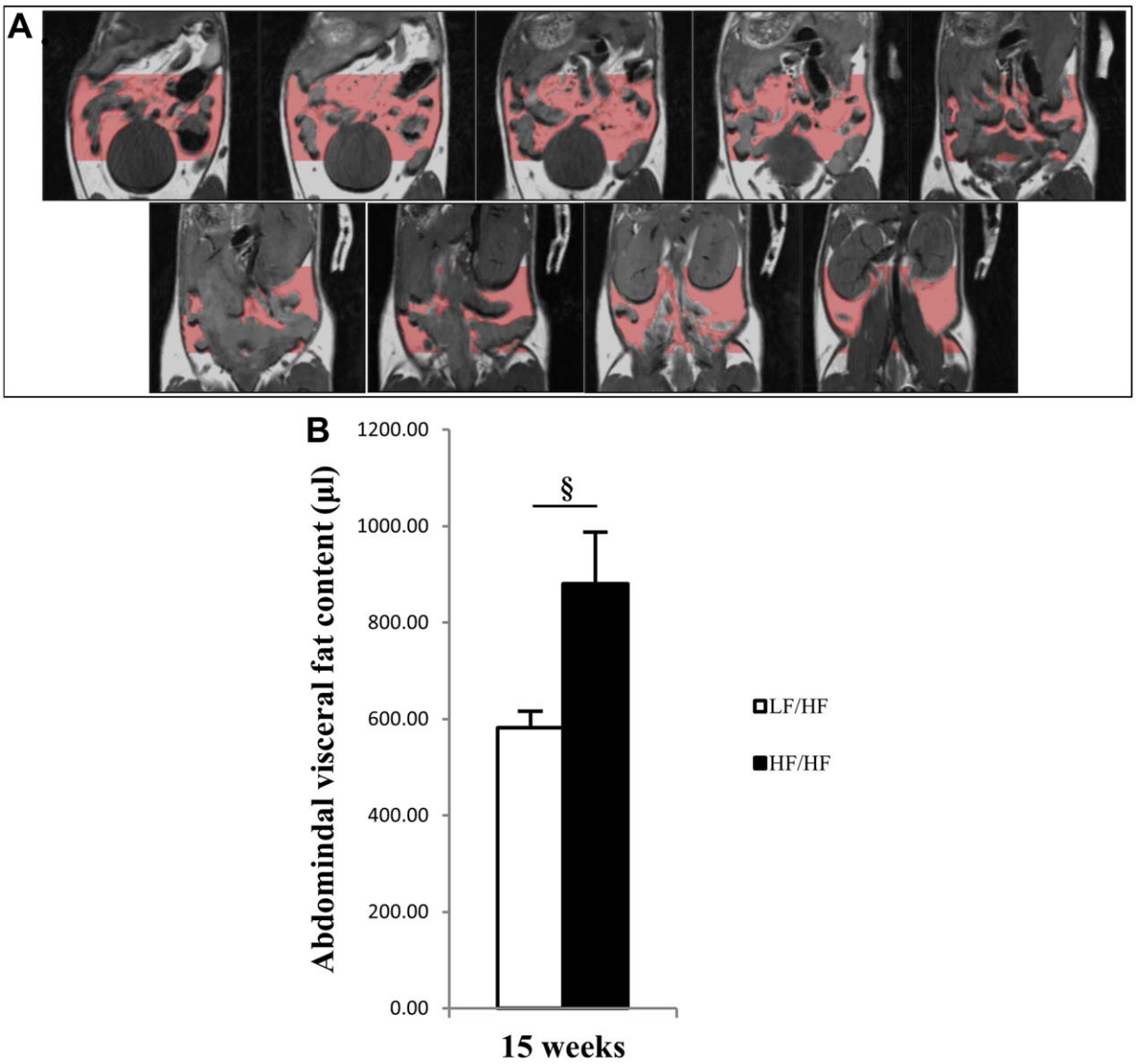

Figure 1 (**A**) Abdominal VAT was determined in the three-dimensional region from half the size of the left kidney to half the size of the bladder (from left to right, from top to bottom). (**B**) Mean (± SEM) abdominal visceral fat content (μl) per group. A tendency for differences in VAT content between the groups is indicated with § $P \leq 0.1$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Hepatic lipid content and mitochondrial function

To investigate if similar effects are found in liver, hepatic lipid content was determined using ^{1}H-MRS. Typical spectra are shown in Figure 4. In contrast to the elevated lipid content in the heart, hepatic fat content tended to be reduced in mice exposed early to HF at 15 weeks of age (LF/HF vs. HF/HF: $4.50\% \pm 0.43\%$ vs. $3.64\% \pm 0.18\%$ of water signal, $P = 0.07$). These results were confirmed by *ex vivo* analysis of hepatic fat content in a parallel group of mice killed at 12 weeks of age (LF/HF vs. HF/HF: 271.67 ± 49.21 vs. 169.07 ± 16.18 μmol g^{-1} of liver, $P = 0.06$). Despite the reduced hepatic fat content in young mice, mitochondrial respiration on glutamate was significantly reduced in mice exposed early to the HF diet at 12 weeks of age (State 3: 1125 ± 96 vs. 902 ± 41 pmol(s $\times$ mg)$^{-1}$, $P = 0.05$). No differences in mitochondrial respiration with palmitoylCoA as a substrate were observed between groups, and mitochondrial DNA copy number was not affected by early exposure to the HF diet (Table 2).

Postprandial lipid handling and *de novo* lipogenesis

As both cardiac and hepatic lipid content may be affected by dietary fat handling, we first investigated cardiac and hepatic dietary lipid retention. Cardiac lipid retention was similar in both groups (LF/HF vs. HF/HF: $0.21\% \pm 0.06\%$ vs. $0.20\% \pm 0.07\%$ of bolus, NS, Figure 5A). However, early exposure to a HF diet resulted in a marked 3.1 fold reduction of hepatic lipid retention after a meal (LF/HF vs. HF/HF: $2.73\% \pm 0.85\%$ vs. $0.89\% \pm 0.26\%$ retention from bolus,

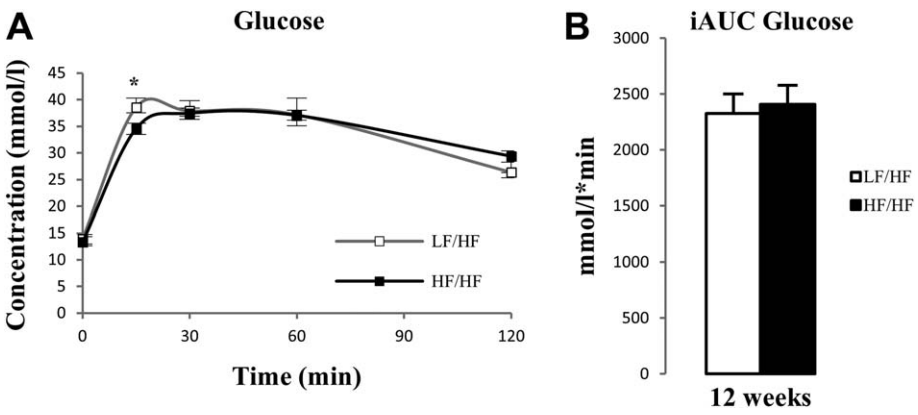

Figure 2 (**A**) Mean (± SEM) plasma glucose concentrations during the glucose tolerance test. Only 15 min after the start of the IPGTT, a significant difference in plasma glucose concentration was found, indicated with * $P \leq 0.05$. (**B**) Area under the curve (AUC) of the glucose concentration during the ipGTT. Glucose clearance was similar for the LF/HF and HF/HF mice.

$P = 0.04$, Figure 5B). These results are consistent with a reduction in plasma TG clearance upon a lipid load in HF/HF mice. Both groups were subjected to an oral bolus of olive oil and determined postprandial TG plasma concentrations, which were much higher in the HF/HF group compared to LF/HF group (6,753 ± 2,213 vs. 14,367 ± 1,978 mmol l^{-1} min^{-1}, $P = 0.01$, Figure 6). The contribution of DNL to the hepatic lipid content, traced by ^2H_2O, was also determined and tended to be higher in the HF/HF group (LF/HF vs. HF/HF: 4.24% ± 0.82% vs. 7.59% ± 1.38%, $P = 0.06$, Figure 5C).

Expression of LPL and modulators of LPL activity

The reduced dietary lipid retention in the liver can be due to a reduced capacity of the liver to take up dietary lipids or to a reduced clearance in LPL-rich tissue such as adipose tissue. Therefore, we investigated expression of genes involved in these processes in liver and adipose tissue. No difference in hepatic Angtpl4, Angptl3, ApoE, and ApoC3 mRNA expression levels was found between LF/HF and HF/HF mice. Hepatic lipase showed a trend towards lower expression in mice exposed early to the HF diet compared to control mice (HF/HF vs. LF/HF: 0.89 ± 0.08 vs. 1.08 ± 0.06, $P = 0.08$). The expression of LPL, Angtpl4, Gpihbp1 and PPARγ in WAT was similar in both groups (Supporting Information Table S1).

TABLE 1 Basic characteristics

	LF/HF	HF/HF
Body weight (g)	25.48 ± 0.66	27.28 ± 0.70§
Heart weight (g)	0.19 ± 0.01	0.18 ± 0.01
Liver weight (g)	0.96 ± 0.02	0.94 ± 0.06
WAT weight (g)	0.77 ± 0.08	1.00 ± 0.11
Plasma TG (mmol l^{-1})	0.86 ± 0.07	0.82 ± 0.05
Plasma NEFA (mmol l^{-1})	0.40 ± 0.03	0.49 ± 0.07
Plasma glucose (mmol l^{-1})	14.0 ± 0.8	13.4 ± 0.6
Insulin (pmol l^{-1})	120 ± 8	135 ± 14
HOMA-IR	10.4 ± 0.9	10.9 ± 1.1

Results are expressed as means ± SEM for 11 LF/HF mice and 11 HF/HF mice from group 1. WAT, white adipose tissue; TG, triglycerides; NEFA, nonesterified fatty acids; HOMA-IR, Homeostasis Model Assessment-estimated Insulin Resistance, with § indicating a trend (§ = $P \leq 0.1$) between the groups .

Discussion

It is generally accepted that the consumption of fat-rich diets favors the development of obesity and cardiovascular disease, often accompanied by accumulation of ectopic lipids and impaired postprandial lipid handling. An underestimated factor in the development of this phenotype may be the long-lasting influence of the exposure to an obesogenic environment during very early development (*in utero* and during lactation). In the present study we investigated whether exposure to an obesogenic HF diet increases the susceptibility for lipotoxic effects in the next generation. We focused on the effect on glucose tolerance, visceral, hepatic, and cardiac fat, and cardiac function. Mitochondrial function was also determined and effects on postprandial lipid handling were investigated.

We describe for the first time that exposure to a HF diet resulted in major disturbances in dietary lipid handling in offspring with increased postprandial plasma TG concentrations. Because it is well established that postprandial hypertriglyceridemia is a risk factor for cardiovascular disease (21), exposure to a HF diet during early development may be an important risk factor for morbidity and mortality later in life. It is important to note that fasted TG plasma concentrations were similar in both groups, showing the added value of postprandial plasma sampling in investigating metabolic disturbances.

HF feeding resulted in an altered pattern of ectopic fat storage with a tendency for reduced hepatic lipid content and significantly increased cardiac lipid content in offspring at week 15 of age. In general, elevated hepatic lipid content is recognized to be a

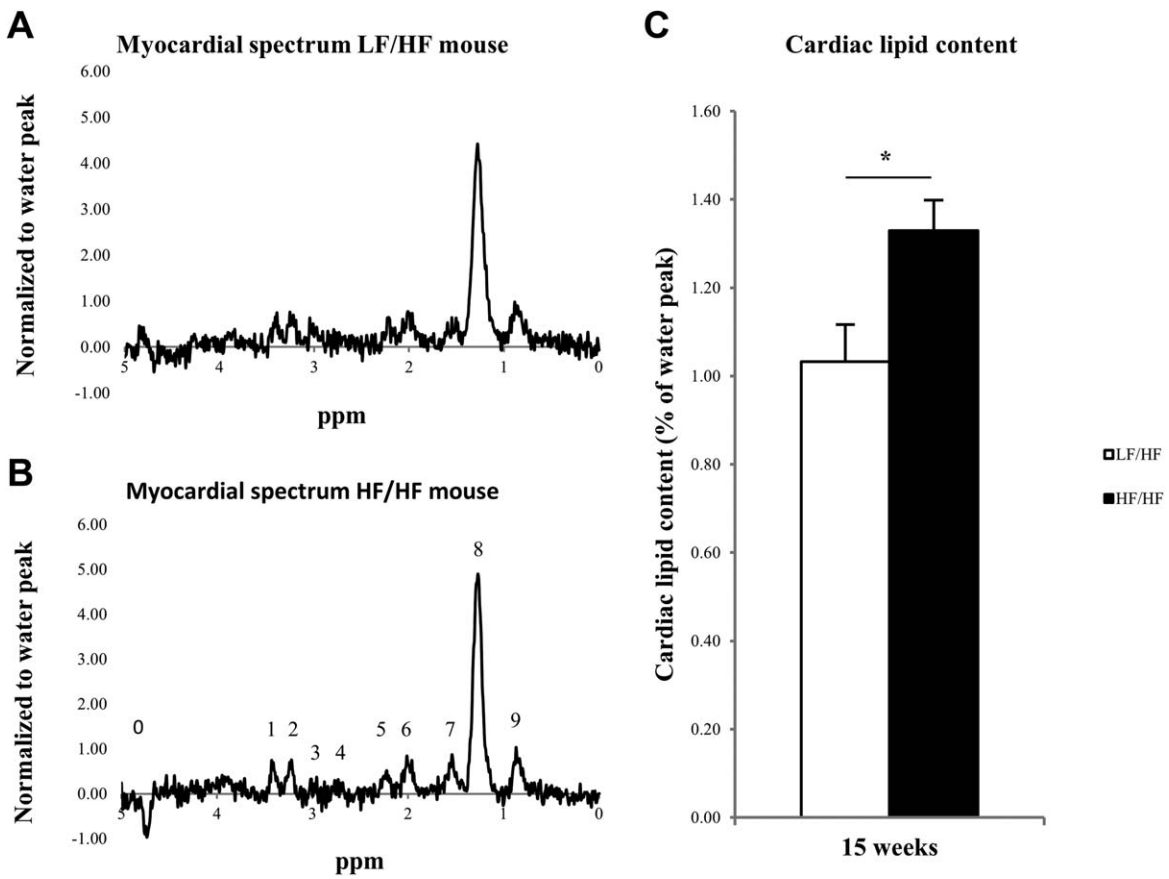

Figure 3 Representative myocardial ^{1}H-MR spectrum obtained from (**A**) a LF/HF mouse and (**B**) a HF/HF mouse. Metabolite peaks were assigned as described previously (19,20). 0. residual (suppressed) water signal, 4.7 ppm; 1. taurine, 3.38 ppm; 2. carnitine, 3.21 ppm; 3. creatine-C**H**$_3$, 2.99 ppm; 4. TG CH=CH-C**H**$_2$-CH=CH, 2.72 ppm; 5. TG C$_\alpha$**H**$_2$COO, 2.20 ppm; 6. TG C**H**$_2$-CH=CH-C**H**$_2$, 2.0 ppm; 7. TG C$_\beta$**H**$_2$CH$_2$COO, 1.55 ppm; 8. TG –(C**H**$_2$)$_n$-, 1.26 ppm; and 9. TG-C**H**$_3$, 0.85 ppm. (**C**) Mean ± SEM myocardial lipid content of mice. Significant differences between the dietary groups are indicated with * $P \leq 0.05$.

determinant in the development of insulin resistance and diabetes, and a reduced liver fat content would be considered a beneficial adaption. However, the reduction in hepatic fat was associated with a disturbed TG clearance and severely decreased hepatic lipid retention from a meal in the HF/HF group, which is probably the underlying reason for the low liver fat content in the current study. Consequently, a low liver fat content is most likely a reflection of disturbances in postprandial TG clearance rather than a reflection of good metabolic health.

When the liver is taking up lipids in the postprandial state, next to the action of hepatic lipase, TG uptake in the liver largely depends on the clearance of chylomicron particles by LPL in adipose tissue or skeletal muscle. To investigate the origin of the impaired TG clearance, we explored mRNA expression of PPAR-γ and LPL as well as known LPL modulators such as Angptl4, Gpihbp1 in WAT and expression of liver-specific LPL modulators (angiopoietin-like proteins Angtpl3, Angtpl4 and apolipoproteins ApoE, ApoC3) as well as hepatic lipase. We found a tendency for reduced mRNA expression of hepatic lipase in HF/HF mice, which may partially underlie the decreased uptake of dietary lipid during a meal and may contribute to the elevated plasma TG concentrations after an oral lipid load in HF/HF mice.

Next to these disturbances in postprandial TG clearance, the HF/HF group shows a tendency for elevated hepatic DNL. DNL is known to be elevated in animal models of NAFLD and in humans with NAFLD and may contribute to the development of a fatty liver in the long term. In a study investigating the origin of hepatic fat in non-alcoholic steatohepatitis (NASH) patients' DNL accounted for 20-30% of fatty acids in the liver (22). Future, more prolonged studies will have to investigate whether in the long term this pathway will actually outweigh the diminished dietary lipid uptake in the liver and whether this will lead to an increased hepatic fat content in the offspring of the HF-fed mice.

In addition to the postprandial hypertriglyceridemia, which represent a cardiovascular risk factor, non-invasive MR imaging revealed that early exposure to a HF diet resulted in a tendency for elevated VAT. It is known that visceral adiposity is associated with and even predicts the development of coronary heart disease, (23) hypertension (23) and type 2 diabetes (24), further suggesting that early exposure to a HF diet results in a less preferable metabolic phenotype. The fact that the fat content of the maternal diet is a strong determinant for later metabolic health is of particular relevance as the typical Western diet contains high amounts of fat and is widely consumed, also by women of child-bearing age. Future research

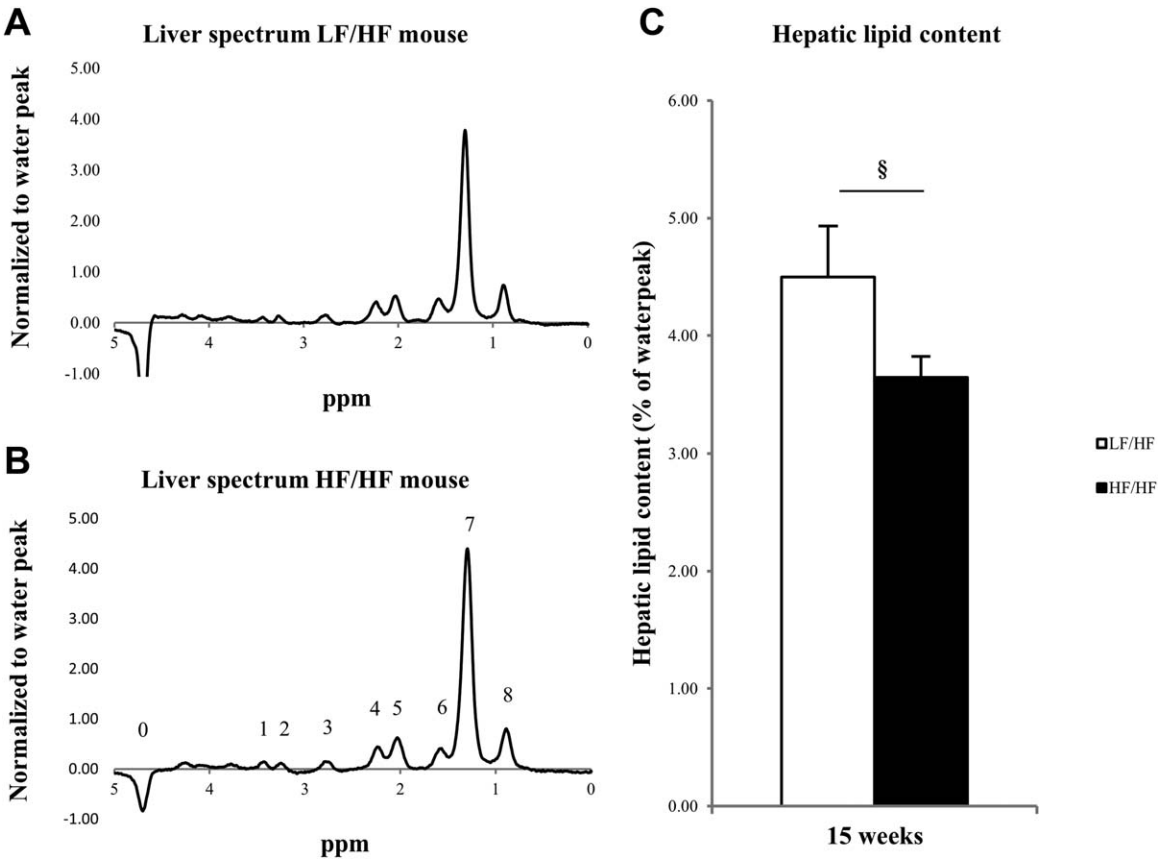

Figure 4 Representative hepatic ^{1}H-MR spectrum obtained from (**A**) a LF/HF mouse and (**B**) a HF/HF mouse. Metabolite peaks were assigned as described previously (19,20). 0. residual (suppressed) water signal, 4.7 ppm; 1. taurine, 3.38 ppm; 2. carnitine, 3.21 ppm; 3. TG CH=CH-C**H**$_2$-CH=CH, 2.72 ppm; 4. TG C$_\alpha$**H**$_2$COO, 2.20 ppm; 5. TG C**H**$_2$-CH=CH-C**H**$_2$, 2.0 ppm; 6. TG C$_\beta$**H**$_2$CH$_2$COO, 1.55 ppm; 7. TG –(C**H**$_2$)$_n$-, 1.26 ppm; and 8. TG-C**H**$_3$, 0.85 ppm. (**C**) Mean ± SEM hepatic lipid content of mice. A tendency for differences between the dietary groups is indicated with § $P \leq 0.1$.

should further investigate the mechanism underlying the identified derangements in postprandial lipid partitioning in order to be able to prevent the development of postprandial hypertriglyceridemia induced by early exposure to a HF diet.

Contrary to the liver, cardiac lipid content was significantly increased. It is well known that cardiac tissue is able to take up circulating TG via the action of cardiac LPL. Therefore, unlike the liver, the heart is not dependent on the clearance of chylomicrons

TABLE 2 Hepatic and cardiac mitochondrial respiration levels [pmol (s × mg^{-1})$^{-1}$]

Liver	LF/HF	HF/HF	Heart	LF/HF	HF/HF
Glutamate-supported respiration			Pyruvate-supported respiration		
State 3	1124.51 ± 95.52	901.76 ± 40.69*	State 3	3502.23 ± 355.07	3649.58 ± 222.57
State 4	254.88 ± 21.53	217.27 ± 14.06	State 4	667.40 ± 41.90	691.71 ± 34.57
RCR	4.46 ± 0.22	4.24 ± 0.23	RCR	5.17 ± 0.34	5.30 ± 0.26
PalmitoylCoA+carnitine-supported respiration			PalmitoylCoA+carnitine-supported respiration		
State 3	585.26 ± 82.73	570.94 ± 82.41	State 3	2915.03 ± 249.06	3218.72 ± 221.94
State 4	262.90 ± 42.12	223.17 ± 15.87	State 4	654.05 ± 77.60	565.94 ± 34.40
RCR	2.28 ± 0.22	2.46 ± 0.20	RCR	5.03 ± 0.60	5.83 ± 0.46

Results are expressed as means ± SEM for 11 LF/HF mice and 11 HF/HF mice. ADP-stimulated (state 3) respiration was induced by the addition of ADP; state 4 respiration was attained upon addition of the ATPsynthase inhibitor oligomycin. Additionally, RCR (respiratory control ratio, S3/S4) respiration is presented, with * indicating statistical significance (* = $P \leq 0.05$) between the groups.

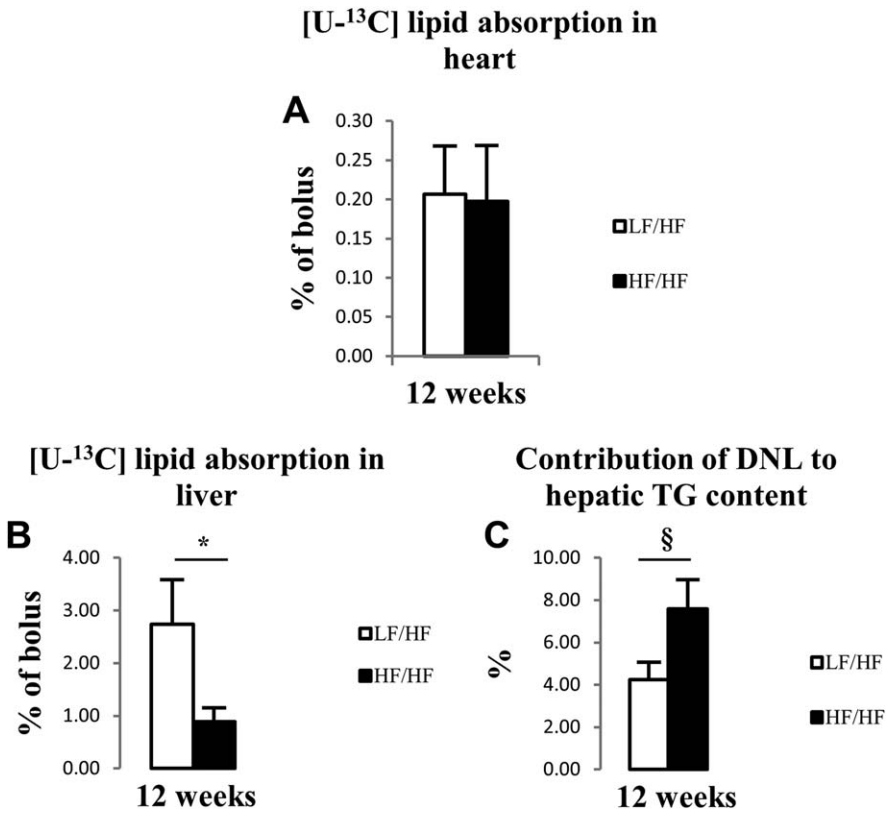

Figure 5 Mean ± SEM of (**A**) cardiac and (**B**) hepatic [U-^{13}C] lipid absorption and contribution of *de novo* lipogenesis to hepatic TG content. No difference in cardiac lipid absorption after a meal was found, but HF/HF mice showed significantly reduced hepatic lipid absorption after a meal ($P = 0.04$) compared to the LF/HF at 12 weeks of age. (**C**) A trend ($P = 0.06$) for elevated contribution of DNL to hepatic TG content was found in the HF/HF group compared to the LF/HF group. Significant differences between the dietary groups are indicated with §$P \leq 0.1$ and *$P \leq 0.05$.

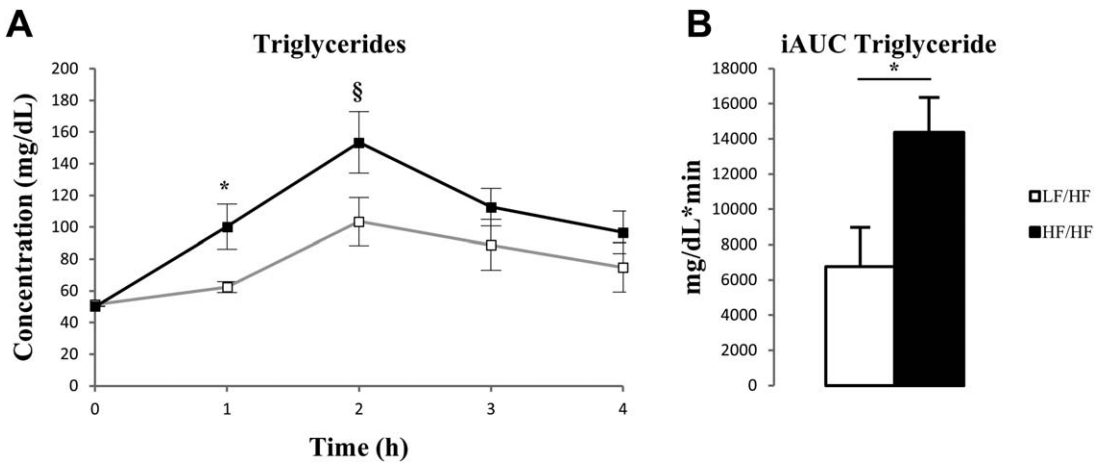

Figure 6 (**A**) One hour after administration of the bolus, a significant difference in plasma TG concentrations was found between the HF/HF and LF/HF group during the TG tolerance test, performed at 12 weeks of age. (**B**) Incremental area under the curve (iAUC) is significantly higher ($P = 0.01$) in the HF/HF mice compared to the LF/HF mice. Significant differences between the dietary groups are indicated with §$P \leq 0.1$ and *$P \leq 0.05$; means ± SEM are shown.

by other LPL-rich tissues (like skeletal muscle and adipose tissue) for TG uptake. The increased cardiac lipid content thus most likely is due to the elevated plasma TG levels observed postprandially. Moreover, it has been suggested that cardiac lipid accumulation may hamper cardiac function by so-called lipotoxic mechanisms (25). However, lipid accumulation in tissue does not necessarily lead to lipotoxicity and tissue dysfunction when the excess lipids are properly stored into lipid droplets. In this study, no compromised mitochondrial function and cardiac function were observed, despite the changes in cardiac lipid content. It is important to note that the increment in cardiac lipid content in the current study is modest when compared to genetic animal models where cardiac lipid content is often severely manipulated, which is then associated with cardiac dysfunction (26-28). The outcome of the present study is in line with earlier findings in rat (29) and men (30,31), showing that a moderate, but physiological change in cardiac fat content is not a strong determinant of cardiac function.

With the current design, we could not distinguish between the effects of maternal obesity independently from the maternal diet. Maternal obesity alone may also be a risk factor for impaired metabolic health [e.g., Ref 32]. Another study limitation is that the measurements were limited to male offspring during young adulthood and future studies will have to determine the effects in female mice and investigate the longer term effects.

In summary, early exposure to a HF diet caused yet unrecognized adaptations resulting in delayed postprandial TG clearance, imposing an increased cardiovascular risk on offspring of HF-fed mice. Impaired TG clearance may in turn be underlying the modest hepatic lipid retention from a meal in the HF/HF mice, resulting in the initially low hepatic fat content, and at the same time, the high postprandial TG levels may favor cardiac lipid accumulation. Future studies are needed to investigate the longer term effects. The stimulation of DNL and visceral fat storage let us anticipate that on the longer term, hepatic fat content will be increased in the group of early HF exposure. Other studies on prenatal HF exposure have often reported elevated hepatic fat content. Future studies are needed to confirm these mechanisms in the human setting, opening new avenues to combat postprandial hypertriglyceridemia caused by early HF exposure. O

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